

IMMUNOLOGICAL RESEARCH ON BRUCELLOSIS AND HUMAN  
IMMUNIZATION AGAINST THIS INFECTION BY MEANS OF  
AN ATTENUATED LIVE VACCINE

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Preliminary Remarks

The aim of our research has been the elaboration of an effective vaccine for human immunization against the melitensis type of brucellosis. The problem in question was resolved successfully, but it required extensive preliminary experimental research in order to reveal the nature and the characteristics of the immunity against brucellosis and to find the most rational type of vaccine. Therefore let us summarize first of all our experimental studies which have motivated the immunization method proposed by us.

The Problem of the Spontaneous Cure of Brucellosis

As is well known, human brucellosis as well as brucellosis in domestic animals manifests itself as a chronic infection, of undetermined duration, quite inclined to slow development, with frequent relapses in man. It is also well known that brucellosis by analogy with other chronic infections and in particular tuberculosis is characterized by the fully explained state of pre-immunization, that is, a marked resistance to superinfection. Little light, however, has been shed upon the problem of the spontaneous cure of brucellosis. One does not know, either, if this spontaneous cure is accompanied by sterile postinfectious immunity.

To solve these last problems which are very important for the understanding of the nature of the antibrucellar immunity, we have undertaken a series of experimental research studies on brucellosis in animals of different species. For this purpose we infected subcutaneously certain parts of animals of a given species with cultures of Br. melitensis or Br. abortus, and we sectioned them at different periods after their infection, while examining them very carefully by the bacteriological and biological method (passages on guinea pigs and mice) in order to show in a precise way the presence or absence of the inoculated brucella.

Under the above-mentioned conditions we have carried out corresponding research on ewes, guinea pigs and rats. We are presenting below the results obtained during this research.

A. Tests on Ewes

The problem of spontaneous cure in ewes seemed particularly important to study because ewes suffer from melitensis brucellosis in natural conditions and constitute in addition to goats the main depository of this organism for human infection.

Translated by: Mrs. Louise Knutson

Let us note among other things that ewes do not manifest an excessive receptivity toward Br. melitensis. Thus our studies have shown that it is necessary to infect the ewes subcutaneously with a considerable dose (approximately 150,000 organisms) of a very virulent culture of Br. melitensis to produce in the ewes a generalized infection by this microbe.

The results of our research on the duration of melitensis infection in ewes and the spontaneous cure of this infection are summarized in Table 1.

Table 1

The Duration of the Infection and the Spontaneous Cure  
In Ewes Infected with Br. melitensis

Periods of Examination After the Infection (Months)	Number of Ewes Examined	Ewes Found Infected	
		Absolute Number	Percentage
$\frac{1}{2}$ - 1	41	38	93.7
$1\frac{1}{2}$ - 3	21	10	48.0
4 - 12	15	5	33.3
19 - 26	22	5	22.7
31 - 41	8	0	0

As one can see by Table 1, melitensis infection in ewes ends in the last analysis by the obvious spontaneous cure. Nevertheless the liberation of the ewes from Br. melitensis takes place very slowly. Thus most of the ewes free themselves from Br. melitensis at the end of two years after the infection, but the cure of all the ewes manifests itself only during the third year after the infection.

#### B. Tests on Guinea Pigs

As is well known, guinea pigs are exceedingly receptive to Br. melitensis. It is sufficient to note that according to very extensive research by our laboratory, the generalized melitensis infection is produced regularly in guinea pigs by the subcutaneous injection of the minimal dose equal to 5 - 10 organisms of a virulent culture of Br. melitensis. Does spontaneous cure of brucellosis take place in these animals without protection against this microbe? Table 2 answers this question precisely.

Table 2

The Duration of the Infection and the Spontaneous Cure  
In Guinea Pigs Infected with Br. melitensis

Periods of Examination After the Infection (Months)	Br. melitensis Cultures Obtained		Positive cultures of Br. melitensis According to the Periods
	Positive	Negative	
1½ - 3½	64	0	1 - 4 months 100% (64 observations)
4 - 5	5	3	4 - 12 months 42.6% (47 observations)
7 - 9	7	10	
10 - 12	8	14	
13 - 16	3	16	
17 - 20	3	14	13 - 26 months 17% (41 observations)
21 - 26	1	4	

As may be seen from Table 2 the spontaneous cure of melitensis type brucellosis manifests itself without any doubt in most infected guinea pigs in spite of their extreme sensitiveness to Br. melitensis. But as in the case of the ewes, the liberation of the guinea pigs infected with this microbe takes place very slowly, and even two years after infection 17% of the animals still retain Br. melitensis in their internal organs.

However the duration of Brucella infection in guinea pigs depends on the virulence of the culture employed. If for example one infects guinea pigs by means of the attenuated culture of Br. abortus, all the animals free themselves from the infection in 8 to 12 months, even in the cases of infection by very massive doses equal to 1½ billion organisms, as is shown in Table 3.

Table 3

The Duration of the Infection and the Spontaneous Cure in  
Guinea Pigs Infected by Attenuated Cultures of Br. abortus

	Periods of Examination After the Infection (Months)									
	1	1-2	2-3	3-4	5-6	6-7	7-8	8-9	10	12-13
Br. abortus 19, Culture BA (percentage)	100	84	51	39	-	26	13-7	0	-	-
Br. abortus M (percentage)	97	83	57	-	33	-	20	5	4	0

C. Tests on Rats

Rats are rather resistant to Brucella infection. Accordingly, these animals rid themselves more easily of the inoculated brucella and in a shorter length of time, in spite of considerable infectant doses of virulent cultures of brucella. Thus Table 4 shows that rats manifest a spontaneous cure within 4 to 6 months after they have been infected with cultures of Br. melitensis and Br. abortus.

Table 4

The Duration of the Infection and Spontaneous Cure in Rats Infected with Br. melitensis and Br. abortus (Virulent Cultures)

Periods of Examination After the Infection (Months)	Rats Examined and Found Infected	
	Br. melitensis	Br. abortus
1 $\frac{1}{2}$ - 1	14/14 100%	16/12 75%
2 - 3	23/16 70%	25/21 84%
4 - 6	12/0 0%	14/0 0%

Remarks: The nominator - Number of rats examined  
The denominator - Number of rats found infected

Conclusion

Thus we see that the spontaneous cure from brucellosis regularly takes place in the infected animals. It is very important for the problem in question that even animals which are very sensitive to Br. melitensis should rid themselves of this microbe in the majority of cases. However the length of time after which spontaneous cure takes place may vary considerably according to the species and the individual characteristics of the animal on the one hand and the virulence of the culture of brucella employed, on the other.

We have clearly exposed the phenomenon of spontaneous cure from brucellosis in ewes, guinea pigs and rats. Soviet veterinarians have shown the same phenomenon in cows. Formerly one of us also observed it among infected goats (P. Zdrodowski).

As a result we may affirm that brucellosis is spontaneously cured in all

of the infected animals, in spite of the chronic character of this disease. But since the infected animals rid themselves very slowly from the brucella, one must admit that immunity against brucellosis develops also very slowly and not easily. It follows that artificial immunization against brucellosis must present a problem which is all the more difficult.

### The Problem of Post-Infectious or Sterile Immunity Against Brucellosis

The spontaneous cure which we have demonstrated enabled us to pose the problem of post-infectious immunity against brucellosis in the animals rid of brucella. We have carried out corresponding research on guinea pigs.

It is well known that the different types of brucella -- melitensis, bovis and suis -- cause in the infected animals the state of cross pre-immunization. That is why we have undertaken the following tests. We infected the guinea pigs subcutaneously with massive doses of the attenuated culture of Br. abortus M and afterwards we re-infected them at different periods of time with 2 to 3 infectant doses of the virulent culture of Br. melitensis (one infectant dose produces always generalized brucellosis in test guinea pigs). One month later the guinea pigs which have been reinfected at different periods of time were sectioned and examined very carefully by the microbiological method with the differentiation of the type of brucella obtained in the cultures according to the bacteriostatic method. At the same time we noted if the type of reinfection was local or generalized and if this reinfection was positive. Table 5 shows the results obtained in the above-mentioned research.

Table 5

The Duration of the Cross-Immunity and its Character in Guinea Pigs Having Been Vaccinated with Br. abortus M and Afterwards Infected with Br. melitensis

The Periods of Infection of Guinea Pigs by Br. melitensis After Vaccination by Br. Abortus M	The Number of Guinea Pigs Vaccinated And Then Infected	The Character of the Infection with Br. melitensis		Character of the Immunity Against Br. melitensis		Summary
		Generalized	Local	Non-Sterile	Sterile	
1 month	30	0	0	26	4	100%
2 months	23	0	3	15	5	87%
3 months	21	0	0	10	11	100%
6 months	26	1	3	4	18	85%
9 months	28	1	5	1	21	79%
12 months	16	5	2	0	9	56%
Test guinea pigs	63	63	0	-	-	0

As may be seen in Table 5, the guinea pigs infected beforehand by the culture Br. abortus M showed an obvious immunity against reinfection by the culture Br. melitensis in 87% to 100% of the cases during the first three months and in 56% toward the end of the year. We notice at the same time that the best guinea pigs show non-sterile as well as sterile post-infectious forms of the acquired immunity. Thus, among the 124 guinea pigs which proved immune during one year of observation, 56 guinea pigs showed the state of pre-immunization and 67 showed sterile post-infectious immunity. We notice finally that during one year of observation in concordance with a gradual elimination of Br. abortus, the cases of pre-immunization in ewes decrease successively and the cases of sterile immunity increase.

Thus, spontaneous cure of brucellosis coincides in guinea pigs with the appearance of sterile immunity. In other words, the disappearing Brucella-infection produces sterile immunity. But Table 5 shows also that the sterile phase of the anti-brucella-immunity is characterized as a relative state and of limited duration. This is why at the end of the year of observation a considerable number of ewes lose immunity. On the other than we see also the marked increase in generalized forms of brucellosis in the longer periods of time after the previous infection with Br. abortus.

Let us notice that we observed sterile post-infectious immunity against brucellosis also in ewes which were infected and spontaneously cured afterwards. We infected six ewes subcutaneously with the culture of Br. melitensis and we re-infected them with the same melitensis culture six months later. After one month the reinfected animals were autopsied and among six ewes examined, the organs of five were found sterile.

### Conclusion

Thus, brucellosis infection is characterized by the state of pre-immunization, that is, by a resistance against superinfections. A spontaneously cured infection leaves in animals the state of sterile immunity against reinfection by brucella. But this state of so-called sterile post-infectious immunity is relative and of limited duration.

### Mechanism of Immunity Against Brucellosis

We have long been persuaded by numerous observations in our laboratory work that the state of immunity against brucellosis does not depend on the antibodies which appear in the infected or vaccinated organism. At the same time, the special research on guinea pigs showed us that the state of pre-immunization, as well as the so-called sterile post-infectious immunity takes place by means of phagocytosis by macrophages and partially microphages. This general thesis divides itself up in the following manner:

(a) The mechanism of immunity against brucellosis, as is manifested in the guinea pigs infected with Br. abortus and later reinfected with Br. melitensis, is linked to the phagocytory process which ends in the complete lysis of the brucella into the macrophages within 43 to 72 hours after reinfection.

(b) The phagocytory reaction observed is itself linked with the general immunological reaction of the organism against infection. It takes place by the cells of the reticulo-endothelial system and by free macrophages of the organism in general and especially on the spot of primary localization of the reinfection or superinfection. (E. Kokorin)

As a result, in guinea pigs infected by *Er. melitensis*, the latter settle upon the spot of infection in a very characteristic manner. This phenomenon occurs particularly in guinea pigs infected subcutaneously with *Er. abortus* 19 and superinfected later intratracheally with the culture of *Er. abortus* 4. This is very well illustrated by Table 6 (according to E. Kokorin).

Table 6

The Fixation of *Er. abortus* 4 in the Lungs after the Intratracheal Superinfection of Guinea Pigs previously Infected by the Culture of *Er. abortus* 19

Periods of Examination After the Superinfection (Hours)	The Cultures of <i>Er. abortus</i> 4 in the Different Organs					
		Lymph Nodes of the Neck	Spleen	Lung	Liver	Blood
1	M	-	-	-	-	-
3	M	-	-	-	-	-
24	M	M	-	-	-	-
48	M	-	M	-	-	-
72	M	M	M	-	-	-
The best Guinea Pigs						
1	M	M	M	-	M	M
3	M	-	M	-	M	M
24	M	M	M	M	M	-
48	M	M	M	M	M	M
72	M	M	M	-	M	-

Note: M = *Er. abortus* 4 obtained in the cultures.

*Er. abortus* 19 produces energetically  $H_2S$ ; *Er. abortus* 4 does not produce  $H_2S$  (the indicated culture).

## Choice of the Rational Type of Vaccine Against Brucellosis

Pre-immunization and sterile post-infectious immunity against brucellosis, although well explained, still remain relative. Special research of our laboratory on this subject has shown that immunity against brucellosis in general must be tested by means of limited doses of the virulent culture of *Br. melitensis*. As a result our laboratory has elaborated the following plan for testing the immunity against brucellosis in guinea pigs: Subcutaneous infection by 2 to 3 infectant doses of *Br. melitensis*, each of which always produces a generalized infection in the test guinea pigs, and the examination of the test guinea pigs sectioned one month after the trial infection.

Since spontaneously cured brucellosis produces sterile immunity in the animals, in our research on a vaccine against brucellosis we have examined above all the immunizing power of the different types of dead vaccine. Still using the test plan for immunity described above, we have shown that by means of repeated injections of dead vaccine, the guinea pigs can be immunized against generalized melitensis infection. However, the dead vaccine produces immunity only in half of the test guinea pigs, and the immunity thus acquired disappears in 75% of the guinea pigs at the end of from 3 to 6 months. These unsatisfactory results were not unexpected for we knew that even post-infectious immunity against brucellosis is relative.

Then we undertook research on live vaccine against brucellosis. With the prospect of elaborating a live vaccine for human immunization, we concluded that we had to look for the necessary varieties among the brucella cultures of the bovis type following theses:

(a) We knew indeed that all types of brucella produce reciprocal immunity and that infection by the bovis type can immunize perfectly against the melitensis type.

(b) At the same time, the bovis type is only facultatively pathogenic for man, and it is known that in the areas where an 's abortion disease occurs the human infections by *Br. abortus* remain almost always inapparent.

Thus one may increase chances of finding cultures innocuous to man among the varieties of *Br. abortus* bovis.

At the same time one may easily summarize the qualities which the varieties of *Br. abortus* must have in order to be used as live vaccine. Obviously these varieties must above all be perfectly innocuous to man while producing in him the inapparent forms of infection; these varieties must also be immunogenic and their qualities stable. It seemed to us also that the examination of cultures of *Br. abortus* on guinea pigs can insure the choice of the varieties in question because of the enormous receptivity of this species of animal to brucellosis.

We tested the different varieties of *Br. abortus* in our collection and we chose two cultures: *Br. abortus* 19-1/A (see footnote 1), American variety widely employed for the vaccination of cows, and *Br. abortus* 4, variety obtained from aborted cows in a district where human brucellosis is not found.

1. E/A - the laboratory number for the variety of *Br. abortus* 19 in our collection of brucella.



The immunizing power of Br. abortus II has already been illustrated above in Table 5. Table 7 characterizes the same power of Br. abortus 19-B/A, showing the efficacy of this variety almost identical with that of culture Br. abortus II.

Table 7

The Duration of Immunity and its Character in the Guinea Pigs Vaccinated by Br. abortus 19 - variety B/A - Then Infected by Br. melitensis

Periods of Examination of Immunity	Number of Guinea Pigs Vaccinated and Examined	Character of Immunity		Summary
		Non-Sterile	Sterile	
1 - 5 days	39	34	0	87%
1 month	32	17	15	100%
2 months	21	4	17	66%
6 months	15	1	12	67%
8 months	10	0	8	80%
12 months	15	0	10	60%
18 months	7	0	1	14%
Test Guinea Pigs	127	-	-	0%

Pathogenic Power of the Chosen Varieties of Br. abortus

Our collaborator, E. Kokorin, has undertaken the histo-pathological study of guinea pigs infected with massive doses ( $1\frac{1}{2}$  billion microbic bodies) at different periods of time after infection, comparing lesions found in those animals with those observed on the test guinea pigs infected with virulent Br. melitensis. As a result of this comparative study, he has found in the guinea pigs infected by the chosen cultures of Br. abortus a moderate hyperplasia of the cells of the reticulo-endothelial system evolving in the internal organs with formation of very few granulomas in the local lymphatic nodes. This hyperplasia persists for about two months after infection and, decreasing little by little, disappears entirely about four months afterward. On the contrary, one observes, in the same periods of time, in the guinea pigs infected with the minimum doses of Br. melitensis a hyperplasia of the reticulo-endothelial system which is widespread and characterized by the formation of very numerous granulomas containing giant cells. Necrobiotic lesions of the cells are also observed in the lymphatic nodes.

Thus the histo-pathological study has quite evidently shown that the varieties of Br. abortus that we have chosen produce in the guinea pigs a benign and transitory infection in spite of the very massive infectant doses. This benign quality of the infection by not very virulent Br. abortus manifests itself in a most evident manner if one compares it with the infection by Br. melitensis in minimal doses which produces in the same conditions in guinea pigs very serious and durable histo-pathological lesions.

Let us add that the two cultures of Br. abortus which we have chosen proved to be equally stabile concerning their qualities.

### Conclusion

All the facts presented above have enabled us to qualify Br. abortus B/A and Br. abortus M. as attenuated, stabile and very immunogenic cultures. That enabled us to test these cultures on men as a live attenuated vaccine against brucellosis.

### Experimentation and Practice of Human Immunization Against Melitensis Brucellosis By Means of the Bovis Type Attenuated Live Vaccine

The varieties of Br. abortus 19 B/A and Br. abortus M, carefully studied on guinea pigs, were first tried on volunteers and then on farmers and slaughter-house workers particularly predisposed to melitensis brucellosis. Later, since 1952, vaccination, after being approved, was adopted as the prophylactic measure in current practice against brucellosis.

At the present time more than 200,000 men have been immunized by means of the vaccine proposed by us. These vaccinations during the first period (1947-1951) were carried out under the direction and control of our laboratory. We are presenting below the results of these tests as the preliminary study of human vaccination against brucellosis. Since 1952 this vaccination has been carried out by the anti-brucella stations in the endemic regions as a practical measure against brucellosis. Further below we shall give the summary results of human vaccination during this latter period.

Everywhere the same lyophilized vaccine (vacuum-dried), produced by our laboratory, was employed (see footnote 1). Before application the dry vaccine is diluted with the sterile physiological solution and it is injected subcutaneously in doses of 150 to 200 million live brucella.

#### A. Preliminary Tests for Human Immunization Against Melitensis Brucellosis by Means of a Live Vaccine of the Bovis Type

We began our research on human immunization by the study of the harmlessness of our vaccine for man. To this end 618 vaccinated persons were checked in dispensaries and examined periodically by clinics. Thus 228 vaccinated persons were observed during 1 to 2 months, 186 up to one year, and 204 during 1 to 2 years.

1. Brucellosis Research Laboratory of the Institute of Epidemiology and Microbiology of the USSR Academy of Medicine

This latter group of 204 men was composed of students of a veterinary institute who in the course of their practice frequented farms where flocks of ewes infected by Br. melitensis were to be found. This group was studied more carefully and systematically.

All of the above mentioned observations showed that immunization by means of a live vaccine of attenuated varieties of Br. abortus proposed by us is perfectly inoffensive for man.

This deduction was proved conclusively by the indisputable and marked decrease of morbidity by melitensis brucellosis among the vaccinated subjects (see below). One must add that among the 204 vaccinated students, 161 blood cultures were made 7, 15 and 30 days after immunization. A hemoculture identical to the variety of infected vaccine was obtained on 15 days after the vaccination (H. Kaitmasova). Thus observations showed that immunization by means of live vaccine of the bovis type produces in man an inapparent vaccinal infection with an episodically transitory hemoculture.

At the same time, observations indicated the appearance in the vaccinated subjects of different immunological reactions which characterize brucella infection in general. Table 8 illustrates these immunological reactions in the group of vaccinated students (according to the results of Madam Kaitmasova).

Table 8

Immunological Reactions in Subjects Immunized by Means of a Live Vaccine of the Bovis Type at Different Periods after Vaccination.

Period After Vaccination	Agglutination Reaction According to Wright	Index of the Opsono-Phagocytary Reaction According to Huddleson	Allergic Reactions According to E. Barnet
7 days	0%	32%	-
17 days	26%	60%	-
1 - 1½ months	66%	51%	40%
4 - 5 months	36%	97%	90%
13 months	20%	47%	92%

Let us add that prolonged observations have shown that positive immunological reactions can occur in 56 to 63% after 2 or 3 years in vaccinated subjects living in endemic areas (that is, being under the influence of inapparent specific infection).

Before speaking of the epidemiological efficacy of the vaccination, it is necessary to note a few important details. All of the vaccinations were made in the endemic areas of melitensis brucellosis upon subjects having negative sero-allergic reactions and receptive to brucellosis. The morbidity of brucellosis in the vaccinated subjects was compared to that among non-vaccinated subjects of the same endemic locality and also having negative immunological reactions. The vaccinations were carried out for two or three months before the season of mis-carriages and the bearing of lambs in the flocks of ewes infected by Br. melitensis. The average indices of morbidity among vaccinated and non-vaccinated subjects for the period from 1947 to 1951 are presented in Table 9.

Table 9

Morbidity of Melitensis-Type Brucellosis Among Subjects Immunized By Means of a Live Vaccine of the Bovis Type, and Non-Vaccinated Subjects

Year	Indices of Morbidity Among Subjects	
	Vaccinated	Non-Vaccinated
1947	0.8%	5.6%
1948	0.4%	6 %
1949	0.5%	29.7%
1950	0.5%	10 %
1951	0.5%	10.4%
	0.5%	12.3%

Thus, as may be seen in Table 9, the summary index of morbidity among vaccinated subjects was equal to 0.5%, and the same index among non-vaccinated subjects was 12.3%.

Let us add that among the 204 vaccinated veterinary students, only one hospitalized case of brucellosis and five ambulatory ones, differentiated by the serological method, were observed during two years, while before vaccination the number of students stricken each year with the very serious forms of melitensis brucellosis was considerable.

One must also note that new cases of brucellosis no longer appeared among slaughterhouse and meat industry workers, as well as on 75% of farms where flocks of infected ewes were to be found.

Thus, in accordance with the research on guinea pigs, immunization of men by means of a live attenuated vaccine of the bovis type has shown itself completely inoffensive and very efficacious at the same time.

B. Human Immunization Against Melitensis Brucellosis by Means of a Live Vaccine of the Bovis Type in Current Practice

At the end of 1951, the results of preliminary tests of human immunization against brucellosis by means of live vaccine were discussed in detail and approved by the special conference which recommended the vaccination to the Ministry of Public Health of the USSR for practical application in the endemic melitensis areas.

Following this recommendation, during 1952-1954 the several thousand workers and inhabitants of endemic localities with flocks of ewes and goats infected with Br. melitensis were immunized by means of the live vaccine proposed by us and prepared by our laboratory.

The vaccination applied on a vast scale appeared everywhere as the inoffensive and clearly efficacious method, since the morbidity among immunized subjects dropped 4.6 - 6.4 and 10 - 10.5 times by comparison with receptive and non-vaccinated subjects.

In particular, convincing results were obtained in the practice of vaccination of subjects especially disposed to infection by Br. melitensis, as is illustrated by Table 10.

Table 10

Morbidity of Melitensis Type Brucellosis Among Vaccinated  
And Non-Vaccinated Subjects Particularly Predisposed to Infection

	Morbidity		Indices of the Efficacy of the Vaccination
	Non-Vaccinated Subjects	Vaccinated Subjects	
Shepherds	76.6%	10.5%	7.3
Farm Veterinarians	73.3%	22.2%	3.3
Farm Workers	61.0%	6.5%	9.2
Owners of Ewes and Goats	49.3%	4.4%	11.2
Other Workers	26.0%	3.5%	7.4

At present, the vaccination proposed by us has taken its place among the principal measures in the prophylaxis of human melitensis brucellosis in the areas of this infection.

Thus, practice has confirmed our experimental research which had predicted the present successful results.

What is the duration of the immunity produced by the vaccine? One may admit that the acquired resistance lasts at least two years if the vaccinated person remains in the endemic areas where natural revaccination undoubtedly occurs. This supposition is affirmed by experimental observation: Guinea pigs immunized by the live vaccine and having lost the acquired resistance recover it very easily under the influence of revaccination even by dead vaccine.

Our investigation of vaccination against brucellosis by means of live vaccine foresees the revaccination of vaccinated subjects who show negative sero-allergic reactions one year after primary immunization. Such a revaccination could be carried out with a live vaccine administered subcutaneously or even cutaneously.